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How does the *in vivo* biliary elimination of drugs change with age? Evidence from *in vitro* and clinical data using a systems pharmacology approach.

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Abbreviations

PK, Pharmacokinetic; PBPK, physiologically based pharmacokinetic; BCRP (ABCG2): breast cancer resistance protein; BE: biliary elimination; GA: gestational age; GFR: glomerular filtration rate; P-gp: P-glycoprotein; MRP2 (ABCC): multidrug resistance-associated protein 2; PMA: post menstrual age.

Abstract

Information on the developmental changes of biliary excretion (BE) of drugs is sparse. The aims of this study were to collate literature data on the pharmacokinetics of biliary excreted drugs used in pediatrics and to apply a Physiologically Based Pharmacokinetic (PBPK) model to predict their systemic clearance (CL) with a view to elucidating age-related changes of biliary excretion. Drug parameters for azithromycin, ceftriaxone, digoxin administered intravenously (IV) and buprenorphine (IV and sublingual) were collated from the literature and used in the Simcyp Simulator to predict adult CL values which were then validated against observed data. The change in CL with age was simulated in the pediatric model and compared to observed data; where necessary, the ontogeny function associated with BE was applied to recover the age-related CL. For azithromycin a fraction of adult BE activity of 15% was necessary to predict the CL in neonates (26 weeks GA) whilst 100% activity was apparent by 7 months. For ceftriaxone and digoxin full BE activity appeared to be present at term birth, for the latter an adult BE activity of 10% was needed to predict the CL in premature neonates (30 weeks GA). The CL of buprenorphine with age was described by the ontogeny of the major elimination pathways (CYP3A4 and UGT1A1) with no ontogeny assumed for the biliary component. Thus, the ontogeny of BE for all four drugs appears to be rapid and attain adult levels at birth or within the first few months of postnatal age.

Introduction

Biliary excretion is a major elimination pathway for many drugs in humans either as the intact parent drug e.g. pravastatin (Henderson et al 2000) or via glucuronidated metabolites e.g. mycophenolate mofetil which may then undergo enterohepatic recirculation (Bullingham et al., 1998). Measuring biliary excretion of drugs in humans is difficult and can be done using a variety of techniques including mass balance faecal recovery of radiolabelled drug and duodenal aspirates. Owing to the invasive nature of the experimental procedures, much of the data is limited to patients having a cholecystectomy, those with a temporary bile shunt (T-tube), or those with bile duct stenosis treated with nasobiliary drainage (Ghibellini et al., 2006). For the aforementioned reasons, it is unlikely, using currently available techniques that the age related change in biliary elimination of drugs will be measured directly in children in the near future.

Consequently, there is very little known about the ontogeny of the biliary excretion of drugs in humans and much of the available information is inferred from animal data or indirect measures in human infants. Gut intraluminal bile acid concentrations are markedly reduced at birth especially in preterm compared to term neonates. However, the latter have around 60% of the adult value and 1 year olds approximately 80% (Balistreri et al., 1983). The total bile acid pool increases progressively from fetus to older infants and reaches adult levels of 3 to 4 g by 2 years of age, but when corrected for body surface area is similar for adults and infants 2 months of age (Heubi et al., 1982). Although biliary elimination appears to develop relatively early, more information on key systems parameters including the ontogeny of transporters are required to substantiate this. The key transporters involved in the active transport of drugs from hepatocytes into the bile canaliculus (P-gp, MRP2 and BCRP) are shown in Figure 1.

There is relatively sparse information on how specific hepatic uptake and canalicular transporters develop with age in pediatrics. However, there is active ongoing research in this area. The ontogeny of hepatic drug transporters and relevance to drug use in pediatrics has been reviewed in this issue (Elmorsi et al., 2015). Much of the current data contains a high degree of uncertainty. Taking P-gp as an example, based on recent data, Pgp mRNA expression is reported to be reduced in neonates, infants and children compared with adults (Mooij et al., 2014), which is in agreement with previous studies (Miki et al., 2005; Fakhoury et al., 2009; van Kalken et al., 1992). However, the same group report no change in protein expression by age (Mooij MG et al., 2015, footnote), which is in agreement with a previous study that excluded neonates (Tang et al., 2007). Another group reports decreased protein expression in neonates, infants and children (Prasad B et al., 2015, footnote). Based on the same information sources, the results for MRP2 are even less certain.

Application of PBPK modelling in support of drug development and regulatory review for both adult and pediatric medicines has expanded significantly in recent years (Leong et al., 2012; Zhao et al., 2011). By accounting for differences in absorption and transit rate, organ size, blood flow, tissue composition and metabolic capacity, the effects of age and formulation on drug PK can be estimated and quantified by PBPK modelling (Johnson and Rostami, 2011; Laer et al., 2009). Such models rely on robust systems data and given the uncertainty in the canalicular transporter ontogeny, this information could not at present be reliably incorporated into a pediatric-PBPK (p-PBPK) model to mechanistically predict the PK of biliary excreted drugs in children. However, the flexible design of such models that allow for different ontogeny profiles for the global biliary clearance element of pediatric drugs, would allow them to be used as a research tool to investigate this issue. Accordingly, the aims of this study were to collate literature data on the pharmacokinetics of biliary excreted drugs used in pediatrics and to apply a Physiologically Based Pharmacokinetic model to predict their systemic clearance (CL) with a view to elucidate the age-related changes of biliary excretion.

Methods

Data collection

Input drug parameters including physicochemical, protein binding and kinetic data for azithromycin and ceftriaxone were collated from the literature after searching MedLine or PubMed. Other data were obtained from Drugbank and PubChem. The input parameters are summarised in Table 1. For buprenorphine, drug related data cited previously were applied (Rowland Yeo et al., 2015, footnote) and the default parameters in the SV-digoxin compound file were used for digoxin with modifications. Clinical studies on age related changes in pharmacokinetics and related parameters were obtained from MedLine using the search terms “Drug” plus “Pharmacokinetics” or “clearance”. Searches were limited to “Humans”, “premature neonates”, “neonates”, “child birth – 18 years”. Article titles and abstracts were screened to restrict the focus of the search to relevant articles and the reference lists of retrieved studies were also scanned to ensure completeness. A summary of the clinical studies used in the analysis is shown in Table 2. All clinical data were either taken directly from the research papers or were extracted from graphs using GetData Graph Digitizer 2.26.

PBPK modelling of biliary elimination

Compound file development

A PBPK model for all compounds was compiled in Simcyp v14.1. The adult renal clearance values for all compounds were entered into the Simulator and for iv azithromycin and ceftriaxone the remainder of the clearance was assigned to biliary using the retrograde calculator to match the adult clinical data on iv clearance. For iv digoxin, the SV-digoxin file within Simcyp was used with minor modification where the biliary elimination was optimized to 30% total elimination based on literature reports (Caldwell and Cline, 1976; Hedman et al., 1990). The buprenorphine compound file contains metabolic information on elimination by CYP3A4, UGT1A1 as well as renal and biliary elimination. To mimic sublingual administration of buprenorphine, the inhaled route was used in the Simulator assuming 80%

of the dose was swallowed. This allowed recovery of the observed bioavailability in adults which is reported to be between 16 to 29% (Elkalder and Sproule., 2005). The percentage elimination assigned to each route for the different drugs are shown in Table 3.

Simulations were run to ensure that the PBPK models developed for each compound were able to predict accurately the PK in adults before undertaking any pediatric simulations.

Pediatric PBPK modelling

The Simcyp Pediatric Simulator contains information on the ontogeny of renal function which is scaled based on predicted GFR in pediatrics divided by typical GFR in adults (120ml/min), this renal value is then used to scale the adult renal clearance value for each compound to that expected for a particular age (Johnson et al., 2006). In all cases no ontogeny was applied to the biliary clearance in the first instance. For buprenorphine an ontogeny is applied both to the CYP3A4 (Upreti and Wahlstrom, 2015) and the UGT1A1 (Abduljalil et al., 2014) components.

For initial iv simulations in azithromycin, ceftriaxone, digoxin and buprenorphine 1000 virtual subjects (10 trials of 100 subjects) were selected covering from full term birth to the maximum age covered by the clinical studies (Table 2). The systemic clearance was plotted against age (y) which was converted to post menstrual age (PMA) in the Microsoft Excel output, the clinical data were then overlaid to allow direct visual comparison of the results.

In the case of azithromycin, the possible ontogeny of biliary elimination in the premature neonates was further investigated using the Simcyp Pediatric Simulator to replicate the concentration-time data in this group (Hassan et al., 2010) by applying a user defined ontogeny to the biliary elimination. For these simulations a full term new-born population 0 – 0.0027y (1 day) was used with a correction applied to account for the reduced GFR in the 26 week GA premature group based on the reference values of Vieux et al., 2010, a 50% reduction in azithromycin renal CL was input into the model to achieve this, all other physiological parameters were left the same Ten trials of 12 subjects with a proportion of

females of 0.5 were set up as the trial design and a number of 'what-if' scenarios were run using the user defined ontogeny corresponding to 10, 15, 20 and 50% of the adult biliary CL value. The simulated mean CL data and 5th and 95th percentiles were compared to the clinical data.

To further investigate the ontogeny of the biliary elimination of digoxin in premature neonates a number of simulations were undertaken by applying the user defined ontogeny to this pathway in order to recover the CL values reported clinically by Hastreiter et al., 1995. Because the GA is not reported and only weight ranges (<1.5 kg and 1.5 to 2.5 kg), the first two groups were combined and a GA of 30 weeks assumed based on the demographic data of Cole et al 2013. Again a new born population 0 – 0.0027y was used in the simulations with a reduction of 40% to the renal clearance of digoxin applied to correct for the lower GRF in this group based on the data of Vieux et al., 2010. Ten trials of 6 subjects, and proportion of females 0.5, were run and the mean observed and predicted CL (L/kg/h) were compared assuming 5, 10 , 20 and 50% of adult biliary elimination.

For the buprenorphine study by Kraft et al., 2008, 10 trials of 12 subjects 0 to 0.0027 y, proportion of females 0.5, were run to replicate the clinical study initially assuming average multiple sublingual doses of 0.0087mg/kg, with the lower and upper reported doses being simulated subsequently. Because of the lack of bioavailability data in neonates no change was made to the adult assumption that 80% of the sublingual dose was swallowed.

Because the drug was administered over a prolonged time period of 47 days in new born term neonates the time-varying physiology model in the Simulator was used to account for changes in physiology occurring during the time course of the study (Abduljalil et al., 2014). For this buprenorphine study the concentration-time data were compared rather than clearance values.

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Statistics

For meta-analysis of adult clinical studies the weighted mean and standard deviation from multiple reports was derived using the following equations:

Overall means (WX) were calculated using equation 1

$$W\bar{X} = \sum_{j=1}^{J} n_j . \bar{x}_j \Big/ \sum_{j=1}^{J} n_j \qquad \text{Equation 1}$$

Where n_j is the number of subjects in the j^{th} study and x_j is the mean value from that study.

Overall SD was calculated using Equations 2 and 3.

$$Overall\,Sum\,of\ Squares = \sum_{j=1}^{J} \left[\left[(sd_j)^2 + (\bar{x}_j)^2 \right] \times n_j \right] - N.(WX)^2 \qquad \text{Equation 2}$$

where sd_j is the standard deviation from the j^{th} study and N is the number of subjects in all studies.

$$Overall\,SD = \sqrt{\frac{Overall\,Sum\ of\ Squares}{N}} \qquad \text{Equation 3}$$

For concentration-time data the predicted 5^{th} and 95^{th} percentiles for the populations were included to ease visual comparison.

Results

The performance of each drug model in adults is shown in Table 3. For all drugs, the predicted clearances were consistent with those reported clinically.

The clearance predictions with PMA for all four drugs are shown in Figure 2. In all of the simulations, no ontogeny was applied for the fraction eliminated directly by biliary excretion. For azithromycin (Figure 2A) the ontogeny of biliary elimination appears to be complete before 8 months of age. However, there are no clinical data on the CL of iv azithromycin from term birth to this age. The CL of azithromycin in premature neonates around 26 weeks PMA is around 0.18 L/h compared to 0.98 L/h in infants aged 6 months. The possible ontogeny of azithromycin biliary elimination in the premature neonates was further investigated using various ontogeny functions within the Simcyp Pediatric Simulator to recover the concentration-time data from the study of Hassan et al., 2011. The results from these 'what-if' simulations are shown in Figure 3, applying an ontogeny to biliary CL corresponding to between 15% of the adult value appears to give the best fit of simulated compared to observed data in the 26 week PMA neonates, assuming a 50% reduction in renal elimination compared to term.

The results for ceftriaxone (Figure 2B) show a lot of variability in the clinical data with mean observed CL values between 0.017 and 0.032 L/kg/h at 32 week GA and between 0.015 and 0.051 L/kg/h at 40 weeks GA. All of these values fall within the range of values predicted assuming no biliary ontogeny and suggest that for this drug the biliary CL is at or near adult values by the time of term birth.

The results for digoxin are shown in Figure 2C. Some of the CL values in the pre-term infant populations show values below those predicted assuming no ontogeny for biliary elimination of this drug. However, by the time of term birth most of the CL values fall within the range of predicted values. Again there is a lot of variability in the clinical data with values ranging from 0.12 to 0.45 L/kg/h in the term neonatal population. To replicate the results in the pre-term

neonates an ontogeny of biliary elimination corresponding to 10 % of the adult level had to be applied in the 30 week GA group in order to recover the CL value of 0.06 L/h/kg seen clinically. This was after applying a 40% reduction to the renal elimination to allow for prematurity, full results are shown in Table 4.

The results for buprenorphine are shown in Figure 2D. The results in children and adults are captured reasonably well. The observed results in the premature neonatal population fall at the bottom end of the predicted results in term new-borns with no biliary ontogeny applied, again suggesting that biliary elimination is at reasonably close to adult values by this stage. Further evaluation of the buprenorphine drug model was undertaken to predict the concentration-time data from the study by Kraft et al., 2008 with no biliary ontogeny applied (Figure 4a,b).

As there was some uncertainty regarding the final doses that individual subjects were titrated to in this study three scenarios were run, median dose, lower dose and upper dose. Only results for median and upper dose are shown. The different cases show that overall the clinical concentration-time data was captured in the simulations assuming no ontogeny for the biliary elimination.

Discussion

In this study we have used a combined 'bottom up' (PBPK) and 'top down' (PK) approach to assess the development of biliary elimination of specific drugs in humans. The biliary ontogeny for each drug is dependent on the transporters involved in their canalicular efflux. Key transporters contributing to the disposition of each drug are shown in Table 5. In general biliary excretion appears to develop rapidly and be at adult equivalent capacity at or soon after birth which is in line with some of the emerging data on canalicular transporter ontogeny in humans. As mentioned previously, the findings of one recent study (Mooij et al., 2015, footnote) indicated that there was no ontogeny for Pgp protein expression in the liver. However, it should be noted that there are conflicting data emerging which shows reduced expression in neonates and infants and compared to adults (Prasad et al., 2015, footnote).

In the current study, the clinical data and PBPK modelling for azithromycin and digoxin suggest that Pgp as a canalicular transporter is at or near adult activity at or just after birth. The same conclusion can be made for MRP2 based on the clinical data from ceftriaxone and azithromycin. This is supported by the protein expression data of Mooij et al., 2015 (footnote) whilst that of Prasad et al., 2015 (footnote) showed significantly ($p<0.05$) reduced MRP2 expression in infants compared to adults. The data on BCRP ontogeny also suggests no correlation between protein expression between neonates and adults (Yanni et al., 2011, Prasad et al., 2015, footnote and Mooij et al., 2015, footnote) and this is reflected in the clinical data for ceftriaxone. The specific transporters involved in buprenorphine biliary excretion have not yet been identified.

In order to further explore the ontogeny of canalicular transporters in premature neonates a number of 'what if' scenarios were run for azithromycin and digoxin in order to capture either the concentration-time or CL data. The ontogeny tool within the Pediatric Simulator allows for a user defined fractional ontogeny for biliary excretion relative to adult. Because the p-PBPK model was built based on data from term neonates onwards it was necessary to

include a correction for renal function and thus renal clearance before simulating in premature neonates. All other factors including body size metrics, liver blood flows and protein expression were left the same in the model so although it cannot be regarded as a true physiological representation of a premature neonate it is close enough for the purpose of these simulations especially because the clearance results are expressed normalised to body weight. For digoxin a Pgp substrate an ontogeny representing around 10% of adult activity had to be applied to capture the clinical data, for azithromycin a 15% adult activity was applied which may reflect the additional MRP2 transport of this drug.

Although an interesting example because of the sublingual as well as iv administration in the examples shown, buprenorphine is perhaps the least useful of the compounds in terms of biliary ontogeny because of its relatively low fraction eliminated by this route. The ontogeny of the other main pathways, CYP3A4 and UGT1A1 are relatively well defined in p-PBPK models (Salem et al., 2014, Upreti et al., 2015, Adbuljalil et al., 2014) and assuming no ontogeny for the biliary component the current drug model was not able to capture the clinical data in the 27 to 32 week PMA group. The ontogeny of both of these enzymes prior to birth is relatively unknown but assuming they are expressed at a lower level than at birth then this would be enough to explain the clinical data without applying additional ontogeny to biliary elimination. To simulate the data of Kraft et al., 2008 for treatment of neonatal abstinence syndrome assumptions had to be made regarding the dose given as only a range was reported in the clinical study. To cover all eventualities three dose levels were simulated, starting with the median dose of 8.7 µg/kg three times a day (tid) but also 4.4 and 13 µg/kg tid representing lower and upper doses. The clinical data was captured by the median and upper dose simulations assuming no ontogeny for the biliary elimination component. Because of the long term nature of the clinical study (up to 46 days) relative to the age of the neonates it was necessary to use the time based changing physiology option in the Simulator which allows for the ‘growth’ of the system parameters with time, this is described in more detail by Abduljalil et al., 2014. The assumption that 80% of the sublingual

dose of buprenorphine was swallowed in adult may not apply to neonates where bioavailability is unknown but may be lower due to drooling. This could also explain some of the over-prediction of the simulated concentration-time profile following the median dose but more likely this is due to the misspecification of dose.

There are a number of limitations of the current study not least of which is the limited number of drugs used in the pediatric age range where the drug is excreted to a significant degree by biliary elimination. However, the current study provides some evidence that biliary elimination appears to be reasonably well developed by the time of birth with a possible ranking of evidence of azithromycin>ceftriaxone>digoxin>buprenorphine. Even in the case of azithromycin the lack of available clinical data in the birth to 6 month age group makes it difficult to conclude exactly when the biliary ontogeny reaches adult levels. The current study does illustrate the potential usefulness of the p-PBPK modelling approach as a research tool in pediatrics where performing direct clinical studies to assess the ontogeny of biliary would not be feasible due to logistical and ethical reasons. Previously we have shown the utility of this methodology for other scenarios where it was challenging to perform studies in assessing metabolic drug-drug interactions in different ages of children (Salem et al., 2013). The latter approach has been taken up in regulatory guidance on DDI assessment in special populations, including pediatrics by both the FDA and EMEA. Such use of PBPK models to extrapolate outside the study populations and experimental conditions required careful attention to a number of issues and confidence that key systems parameters within the PBPK model are correct (Tsamandouras et al., 2015).

Combining the ‘bottom up’ and ‘top down’ approaches and fitting of PBPK models to the clinical data is a useful approach to try and quantify some ‘unknown’ systems parameters, such as canalicular transporter ontogeny in this study, or to better quantify others such as CYP3A4 ontogeny (Salem et al., 2014). Because some of the drugs are substrates for more than one canalicular transporter the information from these can only be interpreted in general terms in relation to their likely ontogeny. Although some information on the ontogeny

of specific transporters in relation to protein expression is now emerging, some of this is contradictory and there is a clear need for more information in this area using validated methodology. Clinical samples should be well characterised in terms of handling and general patient characteristics including relevant genotyping.

Conclusion

Based on limited clinical data the ontogeny of biliary elimination for all four drugs appears to be rapid and reach adult levels at birth or in the first few months of postnatal age. More research is required in this area particularly on the detailed ontogeny of specific canalicular transporters in humans.

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Author Contributions

Participated in research design: Johnson, Jamei, Rowland-Yeo

Performed data collection and analysis: Johnson, Rowland-Yeo

Wrote or contributed to the writing of the manuscript: Johnson, Jamei, Rowland-Yeo

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Footnotes

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b)

- Mooij MG, Van de Steeg E, Wortelboer H, Vaes W, Tibboel D and de Wildt S . Hepatic transporter protein expression in foetuses, neonates and young infants, Oral communication, ESDPPP meeting, Belgrade June 2015.

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Figure Legends

Figure 1. The main human hepatic uptake and bile efflux transporters and their typical substrates. BSEP (ABCB11): bile salt export pump; MATE (SLC47A): multidrug and toxin extrusion protein; MDR1, P-glycoprotein (P-gp; ABCB1): multi-drug resistance 1, P-glycoprotein; MRP (ABCC): multidrug resistance- associated protein; NTCP (SLC10A1): Na+-taurocholate co-transporting polypeptide; OAT (SLC22A): organic anion transporter; OATP (SLCO): organic anion transporting polypeptide; OCT (SLC22A): organic cation transporter; OSTα/β: organic solute transporter.

Figure 2. Change in weight normalized clearance with PMA for (A) azithromycin, (B) ceftriaxone, (C) digoxin and (D) buprenorphine. The light open grey circles represent the simulated individual subjects and the open black symbols are clinical studies as per individual legend, the size of the symbols reflects the size of the clinical study, the error bars are ±SD (where reported). The dashed vertical black line represents full term birth at 40 weeks PMA.

Figure 3. Predicted concentration-time profile for azithromycin in 26 week old preterm neonates assuming (A) 50%, (B) 20%, (C) 15% and (D) 10% of adult biliary elimination activity. The black line is the mean predicted profile and dashed grey lines the predicted 5^{th} and 95^{th} percentiles, the open black circles are the clinical data.

Figure 4. Predicted concentration-time profile of multiple sublingual doses of buprenorphine in term neonates (A) assuming median dose and (B) assuming upper dose. The black line is the mean predicted profile and dashed grey lines the predicted 5^{th} and 95^{th} percentiles, the filled grey circles are the clinical data.

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Table 1. Summary of the PBPK model drug input data for azithromycin, ceftriaxone, digoxin and buprenorphine

	Azithromycin	**Source/Reference**
MW (g/mol)	747.9	-
log P	4.02	Pubchem / Drugbank
Compound type	Monoprotic base	-
pKa1 (acid), pKa2 (base)	8.7	Pubchem / Drugbank
B:P	1	Optimised
fu	0.69	Foulds et al., 1990
V_{ss} (L/kg)	13 (Vd = 16 – 33.1 reported)	Predicted - Full PBPK model Hoffler et al., 1995, Luke et al., 1996
CL_{iv} (L/h)	46.5	Luke et al., 1996
CL_{Renal} (L/h)	8.67	Lalak et al., 1993
Biliary clearance (µl/min per million cells)	9.25	Calculated - Simcyp retrograde model
	Ceftriaxone	**Source/Reference**
MW (g / mol)	554	-
log P	-1.7	Pubchem / Drugbank
Compound type	Diprotic acid	-
pKa1 (acid), pKa2 (base)	3 , 4.1	Pubchem / Drugbank
B:P	0.55	Assumed value
fu	0.05 to 0.42	Concentration dependent. Fukumoto et al., 2009
V_{ss} (L/kg)	0.096	Predicted - Full PBPK model

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CL_{iv} (L/h)	1.30	Meta-analysis
CL_{Renal} (L/h)	0.73	Yuk et al., 1989
Biliary clearance (µl/min per million cells)	1.034	Calculated - Simcyp retrograde model
	Digoxin	**Source / Reference**
MW (g/mol)	781	-
log P	1.26	Pubchem / Drugbank
Compound type	Neutral	-
pKa1 (acid), pKa2 (base)	-	Pubchem / Drugbank
B:P	1.07	Simcyp meta-analysis
fu	0.71	Simcyp meta-analysis
V_{ss} (L/kg)	6.13	Predicted – full PBPK model
CL_{iv} (L/h)	13.46	Simcyp meta-analysis
CL_{Renal} (L/h)	9.66	Simcyp meta-analysis
Biliary clearance (µl/min per million cells)	3.68	Calculated based on 30% – Simcyp retrograde model
	Buprenorphine	**Source / Reference (see footnote c)**
MW (g/mol)	467.64	-
log P	4.82	Avdeef, 2003
Compound type	Ampholyte	-
pKa1 (acid), pKa2 (base)	9.62; 8.31	Avdeef, 2003
B:P	1	Bullingham et al., 1980
fu	0.04	Elkader and Sproule., 2005
V_{ss} (L/kg)	2.7	Bullingham et al., 1980
V_{sac} (L/kg)	1.3	Optimised
k_{in} (h^{-1})	0.64	Optimised

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k_{out} (h^{-1})	0.7	Optimised
Caco-2 $Papp_{A-B}$ 7.4:7.4(10^{-6}cm/s)	66.7	Hassan et al., 2009
Q_{Gut} (L/h)	14.5	Predicted
$CL_{int,u}$ (µl/min per mg)	889	Retrograde approach using CL (IV dosage) from Huestis et al., 2013
CYP3A4	472	Kilford et al., 2009
UGT1A1-population	279	Kilford et al., 2009
UGT1A1-EM	341	Corrected for UGT1A1 phenotype
CL_{Renal} (L/h)	0.535	Bullingham et al., 1980
Biliary clearance (µl/min per million cells)	51	Calculated - Simcyp retrograde model

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Table 2. Summary of clinical studies of iv administered drugs used to compare the simulated data.

Drug	**Age (mean ± SD or range)**		**N (M:F)**	**Dose (mg/kg)**	**Mean CL (L/kg/h) ± SD**	**Reference**
	GA (Weeks)	PMA (weeks or years				
Azithromycin	26.1	26.1 w	12 (5M)	10 (SD)	0.18±0.05	Hassan et al., 2010
Azithromycin	25.6	26 w	44 (10M)	20 (SD)	0.21±0.08	Viscardi et al., 2013
Azithromycin	-	0.66 – 1.8 y	8 (8M)	10 (SD)	0.98±0.29	Jacobs et al., 2005
Azithromycin	-	2.2 – 4 y	8 (8M)	10 (SD)	1.06±0.29	Jacobs et al., 2005
Azithromycin	-	7 – 11 y	8 (8M)	9.8 (SD)	0.96±0.42	Jacobs et al., 2005
Azithromycin	-	12 – 15 y	8 (8M)	8 (SD)	0.71±0.23	Jacobs et al., 2005
Ceftriaxone	31.7	31.7 ± 4 w	39 (??)	50 (SD)	0.0168±0.0072	Mulhall et al., 1985
Ceftriaxone	31.7	31.7 ± 4 w	39 (??)	50 (MD)	0.032±0.0066	Mulhall et al., 1985
Ceftriaxone	-	3.2 ± 2.2 y	21	50 (MD)	0.0179±0.006	Fukumoto et al., 2009
Ceftriaxone	-	0.92 y	5	50 (SD)	0.043±0.011	Scaad et al., 1982

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Ceftriaxone	-	3.9 y	5	50 (SD)	0.043±0.009	Scaad et al., 1982
Ceftriaxone	Term	0.02 - 2 y	30	50 (MD)	0.051±0.024	Steele et al., 1983
Ceftriaxone	Term	0.02 - 2 y	30	75 (MD)	0.055±0.018	Steele et al., 1983
Ceftriaxone		0.003-0.02	24	50 (MD)	0.0210±0.008	Hayton et al., 1986
Ceftriaxone		0.025 – 0.08y	10	50 (MD)	0.0560±0.04	Hayton et al., 1986
Ceftriaxone	Term	0.54 - 1 y	11	50 (MD)	0.0555±0.024	Hayton et al., 1986
Ceftriaxone	-	1 - 6 y	8	50 (MD)	0.0447±0.009	Hayton et al., 1986
Ceftriaxone	Prem	3.2 d PNA	10	50 (MD)	0.06±0.036	McCracken et al., 1983
Ceftriaxone	Prem	6.7d PNA	3	50 (MD)	0.044±0.006	McCracken et al., 1983
Ceftriaxone	Term?	2.8d PNA	9	50 (MD)	0.11±0.012	McCracken et al., 1983
Ceftriaxone	Term?	22.5d PNA	4	50 (MD)	0.096±0.006	McCracken et al.,1983
Ceftriaxone	Term	0.44 – 5.6 y	5	75 (LD)	0.07± 0.013	Nahata et al., 1986
Ceftriaxone	Term	0.44 – 5.6 y	5	50 (MD)	0.038± 0.007	Nahata et al., 1986
Digoxin	Prem	0 – 0.25 y	40	0.003	0.045	Hastreiter et

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						al., 1985
Digoxin	Prem	0 – 0.25 y	32	0.0046	0.08	Hastreiter et al., 1985
Digoxin	Term	0 - 0.25 y	25	0.0061	0.102	Hastreiter et al., 1985
Digoxin	Term	0.25 - 1 y	15	0.0076	0.186	Hastreiter et al., 1985
Digoxin	-	1 – 5 y	19	0.0064	0.222	Hastreiter et al., 1985
Digoxin	-	6 – 10 y	13	0.0032	0.19	Hastreiter et al., 1985
Digoxin	-	11 – 20 y	22	0.0028	0.162	Hastreiter et al., 1985
Digoxin	36	0	10	0.005	0.12	Nyberg et al., 1980
Digoxin	41.9	0.038	10	0.0075	0.18	Nyberg et al., 1980
Digoxin	Term	0.006 – 0.22 y	7	0.017	0.35±0.2	Wettrell et al., 1977
Digoxin	??	0.008 – 0.02 y	5	??	0.055033	Morselli et al., 1975
Digoxin	Term	0.33 – 0.58 y	2	??	0.129711	Morselli et al., 1975
Digoxin	Term	0.083 – 0.92 y	4	??	0.319635	Morselli et al., 1975
Buprenorphine	27 to 32		12	0.003*	0.23 ± 0.07	Barrett et al., 1993

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Buprenorphine	Term	0.007 y	13 (7M)	0.013**	Conc time data only	Kraft et al., 2008***
Buprenorphine	-	5 - 7.5 y	10	0.003	1.36 ± 0.33	Olkkola et al., 1989
Buprenorphine	-	Adult	39	0.3 – 1.2 mg	0.92 ± 0.28	Bullingham et al., 1980 Kuhlman et al., 1996 **Meta-analysis**

GA is gestational age, PNA is post-natal age

*0.003 mg/kg loading dose then 0.00072 mg/kg/h

**initial dose could go to max of 0.039 mg/kg. N.B. this was a sublingual study

***data extracted from poster by Wu et al based on this publication

http://jdc.jefferson.edu/cgi/viewcontent.cgi?article=1023&context=petfp

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Table 3. Fraction of each compound assigned based on literature evidence to be eliminated by direct biliary, renal and hepatic metabolic elimination in adult HV and performance of the model in these subjects

Drug	**Mean percentage eliminated**			**Clearance (L/h) (Mean±SD)**	
	Renal	**Biliary**	**Hepatic metabolism**	**Obs**	**Pred**
Azithromycin	22	78	-	46.5±6.4	45.0±8.9
Ceftriaxone	60	40	-	1.30±0.9	1.32±0.31
Digoxin	65	30	5	13.5±5.9	12.5±2.35
Buprenorphine	1	17	50 CYP3A4 32 UGT1A1	61.4±18.8	55.5±9.93

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Table 4. 'What if' scenarios for the ontogeny of biliary elimination of digoxin in premature neonates 30 weeks GA

Percentage of adult biliary elimination	**CL (L/kg/h) ±SD**	**Percentage drug eliminated renal / Biliary***
50%	0.095±0.03	54/46
20%	0.069±0.02	73/27
10%	0.060±0.02	84/16
5%	0.055±0.02	91/9

*these are the percentages predicted by the simulator

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Table 5. Summary of the transporters involved in the drug canalicular efflux

Drug	**Transporters**	**References**
Azithromycin	PgP (+++) MRP2 (++)	Sugie et al., 2005
Ceftriaxone	MRP2 (+++) BCRP (++)	Kato et al., 2008
Digoxin	PgP (+++)	Greiner et al., 1999
Buprenorphine	PgP (+/-) BCRP (-) MRP2 (?)	Hassan et al., 2009 Tournier et al., 2009

Figure 1

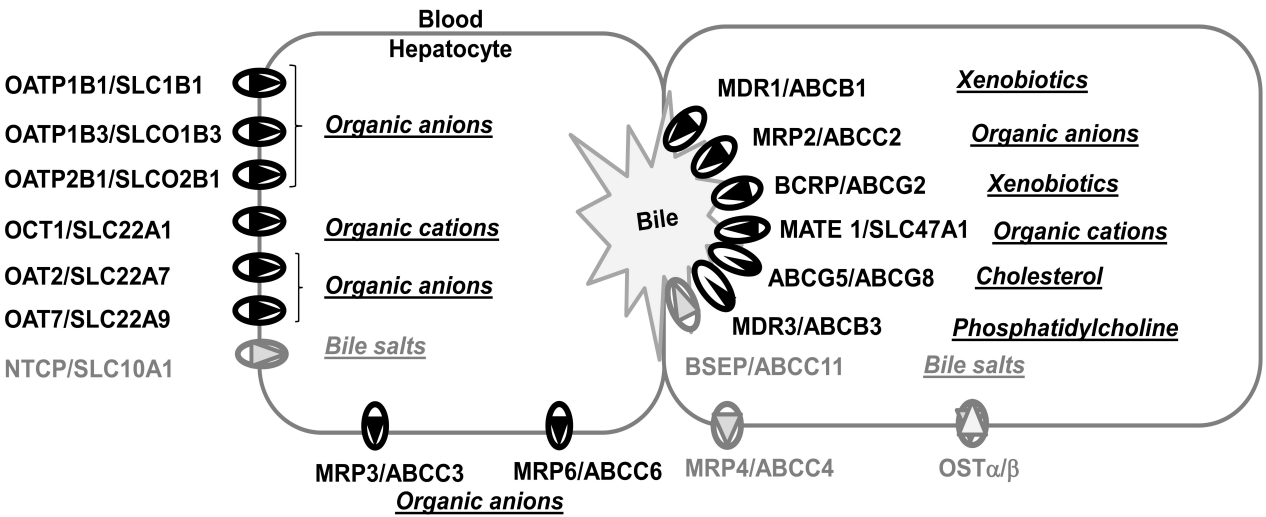

Blood
Hepatocyte
OATP1B1/SLC1B1
OATP1B3/SLCO1B3
OATP2B1/SLCO2B1
Organic anions
OCT1/SLC22A1
Organic cations
OAT2/SLC22A7
OAT7/SLC22A9
Organic anions
NTCP/SLC10A1
Bile salts
MRP3/ABCC3
MRP6/ABCC6
Organic anions
Bile
MDR1/ABCB1
Xenobiotics
MRP2/ABCC2
Organic anions
BCRP/ABCG2
Xenobiotics
MATE 1/SLC47A1
Organic cations
ABCG5/ABCG8
Cholesterol
MDR3/ABCB3
Phosphatidylcholine
BSEP/ABCC11
Bile salts
MRP4/ABCC4
OSTα/β

Figure 2

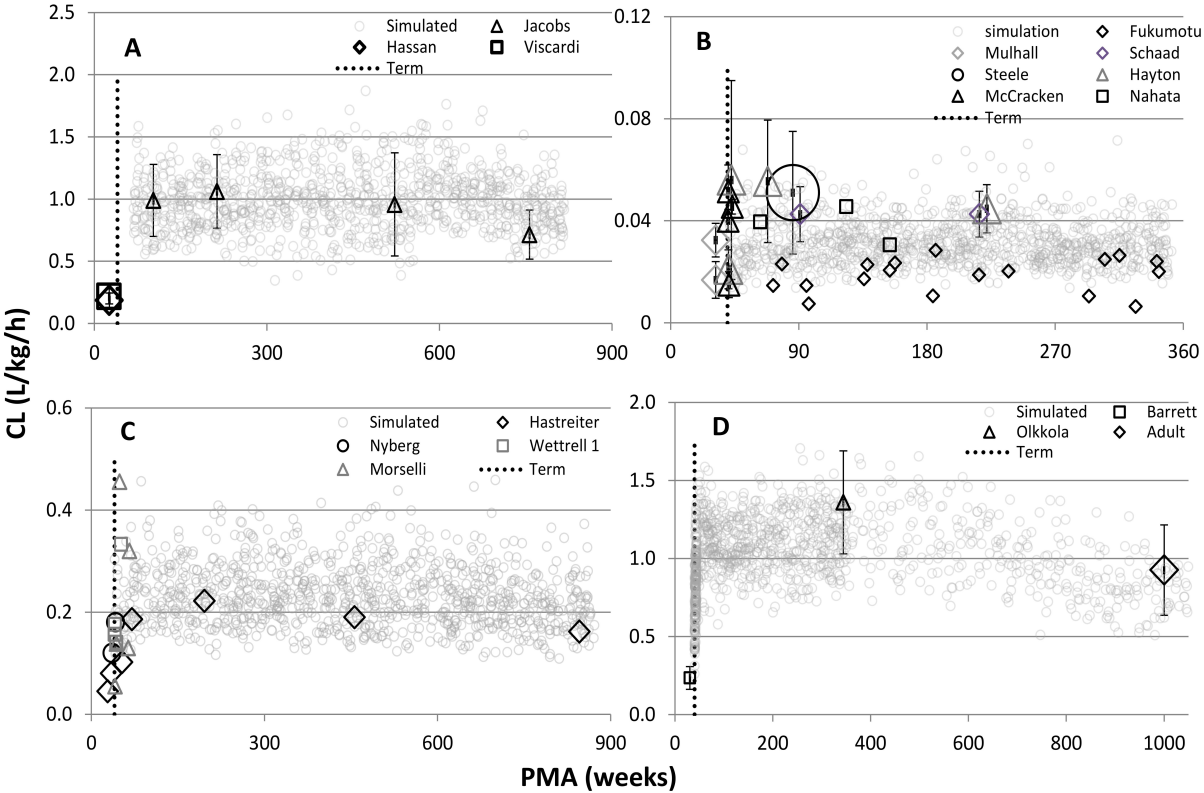

A
Simulated
Jacobs
Hassan
Viscardi
Term
B
simulation
Fukumotu
Mulhall
Schaad
Steele
Hayton
McCracken
Nahata
Term
C
Simulated
Hastreiter
Nyberg
Wettrell 1
Morselli
Term
D
Simulated
Barrett
Olkkola
Adult
Term
CL (L/kg/h)
PMA (weeks)

Figure 3

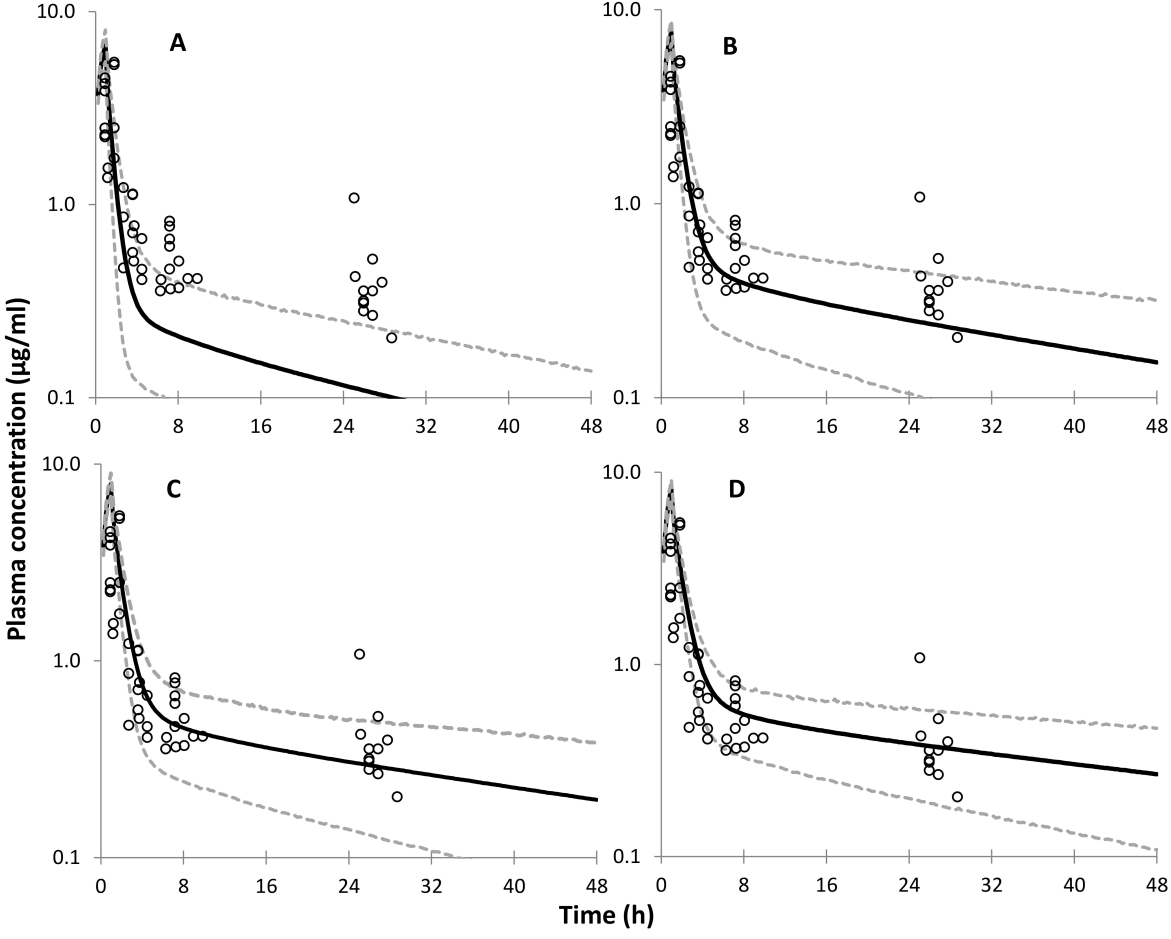
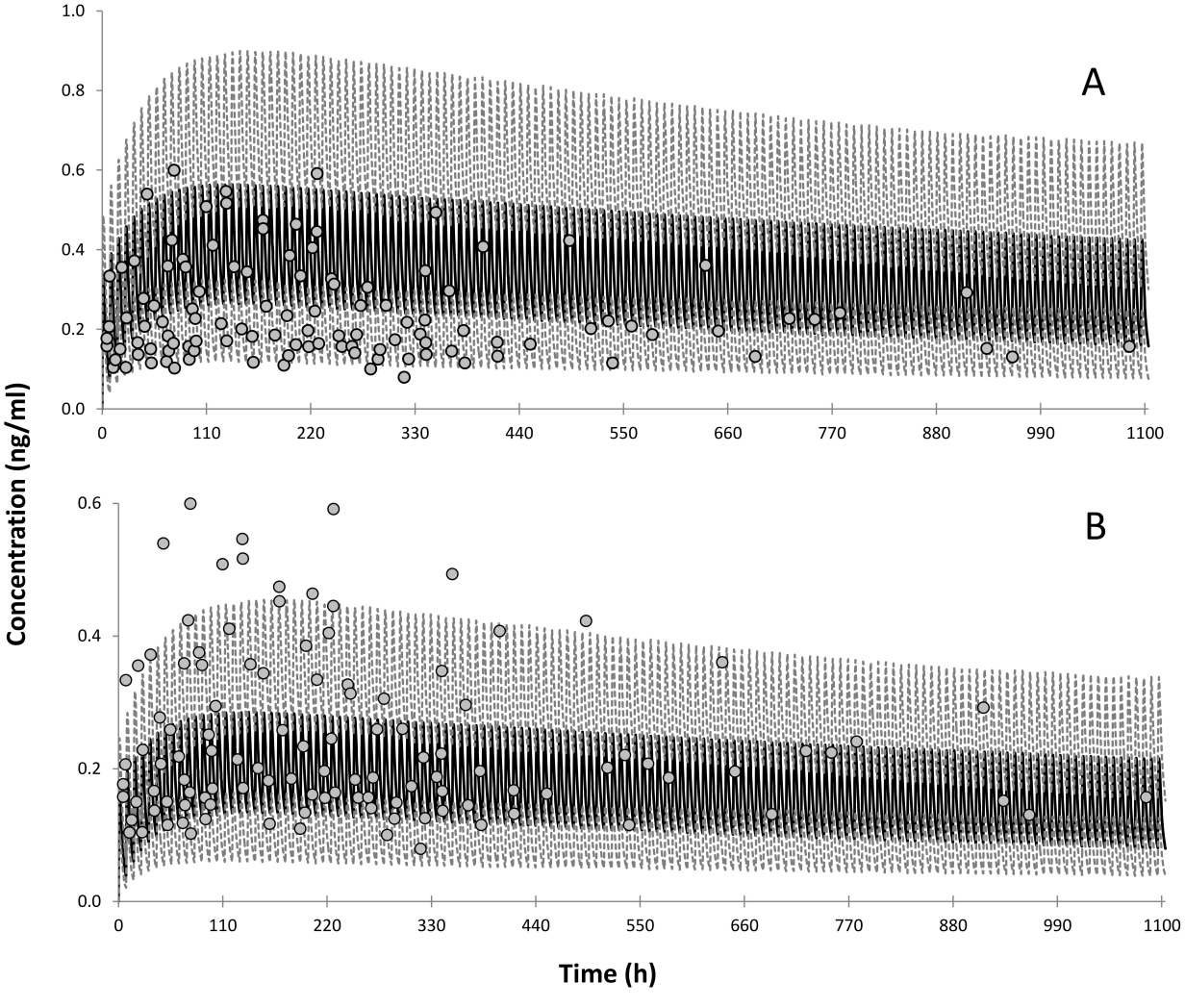

A
B
C
D
10.0
1.0
0.1
0
8
16
24
32
40
48
Plasma concentration (µg/ml)
Time (h)

Figure 4


A
B
Concentration (ng/ml)
Time (h)
0.0
0.2
0.4
0.6
0.8
1.0
0
110
220
330
440
550
660
770
880
990
1100